

Listing of Claims

This listing of claims will replace all prior versions and listings of claims in this application.

1. [Currently Amended] Method for the preparation of a strain of evolved micro-organisms for the production of 1,2-propanediol by the metabolism of a simple carbon source, said ~~which~~ method comprising growing ~~comprises the growth an~~ initial bacterial strain, under selection pressure in an appropriate growth medium comprising containing a simple carbon source, said initial bacterial strain ~~that has undergone~~ comprising a deletion of the gene *tpiA* and ~~the~~ a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate, in order to cause evolution ~~to evolve~~, in said initial strain, of one or more genes involved in the biosynthesis pathway from DHAP to methylglyoxal and then to 1,2-propanediol towards evolved genes having ~~that possess~~ an improved "1,2-propanediol synthase" activity, ~~which resulting then selecting and isolating~~ evolved strain or strains of evolved micro-organisms ~~that possess~~ having an improved "1,2-propanediol synthase" activity ~~are then selected and isolated~~.
2. [Currently Amended] ~~Method according to Claim 1, characterised in that~~ The method of claim 1, wherein the gene involved in the conversion of methylglyoxal into lactate is selected from the group consisting in *gloA*, *aldA* and ~~or~~ *aldB*.
3. [Currently Amended] ~~Method according to either of Claims 1 or 2, characterized in that~~ The method of claim 1, wherein the initial strain ~~has undergone the~~ comprises deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
4. [Currently Amended] ~~Method according to any of Claims 1 to 3, characterized in that~~ The method of claim 1, wherein the initial strain ~~has also undergone the~~ comprises deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.

5. [Currently Amended] ~~Method according to any of Claims to 4, characterized in that~~ The method of claim 1, wherein the initial strain also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
6. [Currently Amended] ~~Method according to Claim 5, characterized in that~~ The method of claim 1, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.
7. [Currently Amended] ~~Method according to either of Claims 5 or 6, characterized in that~~ The method of claim 5, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.
8. [Currently Amended] ~~Method according to Claim 7, characterized in that~~ The method of claim 7, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
9. [Currently Amended] ~~Method according to any of Claims 6 to 8, characterised in that~~ The method of claim 6, wherein the enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme.
10. [Currently Amended] ~~Method according to any of Claims 1 to 9, characterised in that~~ The method of claim 1, wherein one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone are introduced into the evolved ~~strain~~ microorganisms.
11. [Currently Amended] ~~Method according to Claim 10, characterised in that~~ The method of claim 10, wherein one the heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate are from *C. acetobutylicum*.

12. [Currently Amended] ~~Method according to either of Claims 10 or 11,~~
~~characterised in that~~ The method of claim 10, wherein an evolved the modified
evolved strain comprising one or more heterologous genes coding for one or more
enzymes involved in the conversion of acetyl-CoA and acetate into acetone
~~obtained according to either of Claims 10 or 11~~ is grown under selection pressure
in an appropriate growth medium ~~containing~~ comprising a simple carbon source
in order to cause, in said evolved modified evolved strain, the evolution of one or
more genes involved in the conversion of acetyl-CoA and acetate to acetone
towards an improved "acetone synthase" activity ~~The, the~~ second generation of
resulting evolved micro-organisms ~~that possess~~ having an improved "1,2-
propanediol synthase" activity and an improved "acetone synthase" activity are
then selected and isolated.
13. [Currently Amended] ~~Method according to any of the preceding claims,~~
~~characterised in that~~ The method of claim 1, wherein the strain is selected from
the group consisting of a strain of bacterium, a yeast and or a fungus.
14. [Currently Amended] ~~Method according to Claim 13, characterised in that~~
The method of claim 13, wherein the strain is selected from the group consisting
of a strain of Escherichia, in particular E.coli, and Corynebacterium, in
particular C. glutamicum.
15. [Cancelled].
16. [Currently amended] Evolved strain that can be obtained by the method according
to any of Claims 1 to 14.
17. [Original] Strain according to Claim 16, in which the gene *Ipd* has a point mutation
whereby alanine 55 is replaced by valine.

18. [Currently amended] Method of preparation of 1,2-propanediol ~~in which~~ wherein an evolved strain of claim 16 is grown ~~according to either of Claims 16 or 17~~ in an appropriate growth medium containing a simple carbon source, and wherein ~~in which~~ the 1,2-propanediol produced is recovered.
19. [Currently amended] ~~Method according to Claim 18, characterised in that~~ The method of claim 18, wherein 1,2-propanediol and acetone are recovered.
20. [Currently amended] ~~Method according to either of Claims 18 or 19, characterised in that~~ The method of claim 18, wherein 1,2-propanediol and/or acetone are purified.
21. [New] The method of claim 14, wherein the strain is selected among the group consisting of *E. coli*, and *C. glutamicum*.
22. [New] Initial bacterial strain of a microorganism comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate.
23. [New] The strain of claim 22, wherein the gene involved in the conversion of methylglyoxal into lactate is selected among the group consisting in *gloA*, *aldA* and *aldB*.
24. [New] The method of claim 22, wherein the initial strain comprises deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
25. [New] The strain of claim 22, wherein the initial strain comprises deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.
26. [New] The strain of claim 22, wherein the initial strain also contains at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
27. [New] The strain of claim 22, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.

28. [New] The strain of claim 27, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.
29. [New] The strain of claim 27, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
30. [New] The strain of claim 22, selected from the group consisting of a bacterium, a yeast and a fungus.
31. [New] The strain of claim 30, selected from the group consisting of *Escherichia* and *Corynebacterium*.
32. [New] The strain of claim 16, comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal (propanal) into lactate, selected from the group consisting in *gloA*, *aldA* and *aldB*.
33. [New] The strain of claim 16, comprising deletion of the genes *gloA*, *aldA*, *aldB* and *tpiA*.
34. [New] The strain of claim 16, comprising deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.
35. [New] The strain of claim 16, comprising at least one gene coding for an enzyme that favours the metabolism of pyruvate to acetate.
36. [New] The strain of claim 36, wherein the enzyme that favours the metabolism of pyruvate into acetate has low sensitivity to inhibition by NADH.
37. [New] The strain of claim 36, wherein the said enzyme that favours the metabolism of pyruvate into acetate, favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH.

38. [New] The strain of claim 37, wherein the enzyme that favours the metabolism of pyruvate towards the production of acetyl-CoA and NADH is a pyruvate dehydrogenase complex.
39. [[New] The strain of claim 36, wherein the enzyme that favours the metabolism of pyruvate into acetate is an endogenous enzyme.
40. [New] The strain of claim 16, comprising one or more heterologous genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate into acetone.
41. [New] The strain of claim 40, wherein one the heterologous gene or genes coding for one or more enzymes involved in the conversion of acetyl-CoA and acetate is from *C. acetobutylicum*.
42. [New] The strain of claim 16, selected from the group consisting of a bacterium, a yeast and a fungus.
43. [New] The strain of claim 16, selected from the group consisting of *Escherichia*, and *Corynebacterium*.
44. [New] The strain of claim 17, selected from the group consisting of a bacterium, a yeast and a fungus.
45. [New] The strain of claim 17, selected from the group consisting of *Escherichia*, and *Corynebacterium*.
46. [New] Evolved strain that can be obtained by the method of Claim 10.
47. [New] The strain of Claim 46, in which the gene *Ipd* has a point mutation whereby alanine 55 is replaced by valine.
48. [New] The strain of claim 46, selected from the group consisting of a bacterium, a yeast and a fungus.
49. New] The strain of claim 46, selected from the group consisting of *Escherichia* and *Corynebacterium*.